Claims

What is claimed is:

1. An isolated polypeptide having aminopeptidase activity, selected from the group consisting of:

(a) a polypeptide having an amino acid sequence which has at least 50% identity with the amino acid sequence of SEQ ID NO:2;

(b) a polypeptide which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, (ii) its complementary strand, or (iii) a subsequence of SEQ ID NO:1 which encodes a polypeptide fragment which has aminopeptidase activity;

(c) an allelic variant of (a) or (b);

(d) a fragment of (a), (b), or (c), wherein the fragment has aminopeptidase activity; and

(e) a polypeptide having aminopeptidase activity with physicochemical properties of (i) a pH optimum in the range of from about pH 7.27 to about pH 10.95 determined at ambient temperature in the presence of Ala-para-nitroanilide; (ii) a temperature stability of 90% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 60°C in the absence of substrate; and (iii) an activity towards Xaa-para-nitroanilide wherein Xaa is selected from the group consisting of Leu, Glu, Gly, Ala, and Pro.

2. The polypeptide of claim 1, comprising an amino acid sequence which has at least 50% identity with the amino acid sequence of SEQ ID NO:2.

3. The polypeptide of claim 2, comprising an amino acid sequence which has at least 60% identity with the amino acid sequence of SEQ ID NO:2.

4. The polypeptide of claim 3, comprising an amino acid sequence which has at least 70% identity with the amino acid sequence of SEQ ID NO:2.

5. The polypeptide of claim 4, comprising an amino acid sequence which has at least 80% identity with the amino acid sequence of SEQ ID NO:2.

6. The polypeptide of claim 5, comprising an amino acid sequence which has at least 90% identity with the amino acid sequence of SEQ ID NO:2.

7. The polypeptide of claim 1, comprising the amino acid sequence of SEQ ID NO:2 or a fragment thereof.

8. The polypeptide of claim 7, comprising the amino acid sequence of SEQ ID NO:2.

9. The polypeptide of claim 2, which is obtained from aa *Aspergillus* strain.

10. The polypeptide of claim 9, which is obtained from an *Aspergillus oryzae* strain.

11. The polypeptide of claim 1, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, or its complementary strand, or a subsequence thereof which encodes a polypeptide fragment which has aminopeptidase activity.

12. The polypeptide of claim 11, which is encoded by a nucleic acid sequence which hybridizes under medium stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or its complementary strand.

13. The polypeptide of claim 11, which is obtained from an *Aspergillus* strain.

14. The polypeptide of claim 13, which is obtained from an *Aspergillus oryzae* strain.

15. The polypeptide of claim 1, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1, or its complementary strand, or a subsequence thereof which encodes a polypeptide fragment which has aminopeptidase activity.

16. The polypeptide of claim 15, which is encoded by a nucleic acid sequence which hybridizes under high stringency conditions with (i) the nucleic acid sequence of SEQ ID NO:1 or its complementary strand.

17. The polypeptide of claim 15, which is obtained from an *Aspergillus* strain.

18. The polypeptide of claim 17, which is obtained from an *Aspergillus oryzae* strain.

19. The polypeptide of claim 1, which has the following physicochemical properties: (a) a pH optimum in the range of from about pH 7.27 to about pH 10.95 determined at ambient temperature in the presence of Ala-para-nitroanilide; (ii) a temperature stability of 90% or more, relative to initial activity, at pH 7.5 determined after incubation for 20 minutes at 60°C in the absence of substrate; and (iii) an activity towards Xaa-para-nitroanilide wherein Xaa is selected

from the group consisting of Leu, Glu, Gly, Ala, and Pro.

20. The polypeptide of claim 19, which is obtained from an *Aspergillus* strain.

21. The polypeptide of claim 20, which is obtained from an *Aspergillus oryzae* strain.

22. The polypeptide of claim 1, which is encoded by the nucleic acid sequence contained in plasmid pEJG18 contained in *E. coli* NRRL B-21677.

23. An isolated nucleic acid sequence comprising a nucleic acid sequence which encodes the polypeptide of claim 1.

24. A nucleic acid construct comprising the nucleic acid sequence of claim 23 operably linked to one or more control sequences which direct the production of the polypeptide in a suitable expression host.

25. A recombinant expression vector comprising the nucleic acid construct of claim 24, a promoter, and transcriptional and translational stop signals.

26. A recombinant host cell comprising the nucleic acid construct of claim 24.

27. A method for producing the polypeptide of claim 1 comprising (a) cultivating a strain to produce a supernatant comprising the polypeptide; and (b) recovering the polypeptide.

28. A method for producing the polypeptide comprising (a) cultivating a host cell of claim 26 under conditions suitable for production of the polypeptide; and (b) recovering the polypeptide.

29. A method for producing the polypeptide of claim 1 comprising (a) cultivating a homologously recombinant cell, having incorporated therein a new transcription unit comprising a regulatory sequence, an exon, and/or a splice donor site operably linked to a second exon of an endogenous nucleic acid sequence encoding the polypeptide, under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.

30. A method for producing a mutant of a cell, which comprises disrupting or deleting a nucleic acid sequence encoding the polypeptide of claim 1 or a control sequence thereof, which results in the mutant producing less of the polypeptide than the cell.

31. The mutant produced by the method of claim 30.

32. A method for producing a heterologous polypeptide comprising (a) culturing the mutant of claim 31 under conditions conducive for production of the polypeptide; and (b) recovering the polypeptide.

33. A method for producing a hydrolysate from a proteinaceous substrate which comprises subjecting the substrate to a polypeptide of claim 1 and an endopeptidase.

34. The method of claim 33, wherein the hydrolysate is enriched in Leu, Gly, Glu, Ser, Asp, Asn, Pro, Cys, Ala, and/or Gln.

35. The method of claim 33, wherein the hydrolysate is enriched in Gly.

36. A protein hydrolysate produced by the method of claim 33.

37. The protein hydrolysate of claim 36, wherein the hydrolysate is enriched in Leu, Gly, Glu, Ser, Asp, Asn, Pro, Cys, Ala, and/or Gln.

38. The protein hydrolysate of claim 36, wherein the hydrolysate is enriched in Gly.

39. A food product comprising the protein hydrolysate of claim 36.

40. A method for obtaining from a proteinaceous substrate a protein hydrolysate enriched in free glutamic acid and/or peptide bound glutamic acid residues, comprising subjecting the substrate to a deamidation process and a polypeptide of claim 1.

41. The method of claim 40, further comprising subjecting the substrate to one or more unspecific acting endo- and/or exo-peptidase enzymes.

42. A protein hydrolysate obtained by the method of claim 41.

43. A food product comprising the protein hydrolysate of claim 42.

44. A flavor-improving composition comprising a polypeptide of claim 1 and a suitable carrier.

45. A pre-mix for a dough comprising a polypeptide of claim 1 and a baking ingredient.